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Inheritance of Chromosome Heteromorphisms Analyzed by High-Resolution Bivariate Flow Karyotyping

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Summary

The DNA content of the mitotic chromosomes from 10 children and their parents in four families were quantified by bivariate flow karyotyping. In all cases, each chromosome peak in the flow karyotype of the child could be traced to one of the two parents. The measured absolute difference in homologue DNA content between children and their parents averaged 0.8%, or ≈1 Mbp over all chromosome types. This study demonstrates that flow karyotypes of a proband's parents can be an aid in detecting and quantifying the size of de novo deletions that involve heteromorphic chromosome types.

Introduction

Metaphase chromosomes of the same type can differ in DNA content among healthy individuals. These heteromorphisms were first characterized in metaphase spreads stained to produce chromosome banding patterns. Significant differences in the length and intensity of certain chromosomal subregions were observed among normal chromosomes (Geraedts and Pearson 1974; McKenzie and Lubs 1975; see Jacobs 1977 for review). Chromosome heteromorphisms can also be studied using flow cytometry (Green et al. 1984; Harris et al. 1986, 1987; Trask et al. 1989). For this, chromosomes are stained with two DNA-specific fluorochromes with different basepair specificity: Hoechst (HO) or DAPI (adenine-thymidine preference) and chromomycin A3 (CA; cytosine-guanine preference). The fluorescence intensities of individual chromosomes are quantified in a dual-beam flow cytometer to produce a bivariate histogram, or flow karyotype. With this technique, chromosome heteromorphisms can be rapidly and objectively characterized with high accuracy, since chromosomal DNA content can be measured with a precision better than 1%. In addition, heteromorphisms carried by different individuals can be compared in a quantitative manner. In the accompanying paper (Trask et al. 1989), we show that the DNA content and relative base composition of normal chromosome heteromorphisms can be derived from bivariate flow karyotypes. Both microscopic and flow cytometric analyses have shown that the degree and frequency of heteromorphism differ among chromosome types (Geraedts and Pearson 1974; McKenzie and Lubs 1975; Harris et al. 1986; Trask et al. 1989). The acrocentric chromosomes (13–15, 21, and 22) and the chromosomes with heterochromatic regions (1, 9, 16, and Y) are highly heteromorphic. Differences in the size of tracts of repetitive sequences associated with heterochromatin and satellite sequences appear to account for much of the observed heteromorphisms (Jacobs 1977; Harris et al. 1986; Trask et al. 1989). Flow analysis has demonstrated that heteromorphisms of chromosome 21 can differ by as much as 45% in DNA content, while variants of chromosomes 6, 7, and 8 were observed to differ by less than 2% in DNA content (Trask et al. 1989).

Studies of members of the same family have shown that chromosome heteromorphisms, identified by their banding characteristics, can be traced from parent to child. The blocks of repeated DNA sequences localized to heteromorphic chromosome regions appear to be passed as intact units from parent to child (McKenzie et al. 1972; Craig-Holmes et al. 1975; McKenzie and Lubs 1975; Robinson et al. 1976; Verma and Lubs 1976; Kurnit 1979). Indeed, chiasma in synaptonemal

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complexes, evidence of meiotic recombination, have been observed rarely within heterochromatic regions (Kurnit 1979).

In the study described here, we compared the bivariate flow karyotypes of 10 children with those of their parents to determine the fidelity with which chromosome peak position is inherited. This is an extension of studies by Harris et al. (1987), who determined the parental origin of chromosomes resolved by ethidium bromide staining and single-beam flow cytometry. Our studies show that the detection of de novo–occurring chromosome abnormalities by flow cytometry may be improved by comparing parental and patient flow karyotypes. This comparison may be particularly useful, if not necessary, in the quantitative analyses of structural abnormalities involving chromosome types that are highly heteromorphic among normal individuals (Trask et al. 1989).

Material and Methods

Cell Culture and Chromosome Isolation

Peripheral blood (1–5 ml) from normal donors was cultured in the presence of phytohemagglutinin for 72–96 h as described elsewhere (Trask et al. 1989). Cultures were incubated in the presence of colcemid (0.1 μg/ml) for 10 h preceding harvest. Chromosomes were isolated into a buffer that contains $MgSO_4$ for chromosome stabilization and were stained with 3.8 μM HO or 1.4 μM DAPI and 17 μM CA as described elsewhere (van den Engh et al. 1984, 1985, 1988; Trask et al. 1989).

Flow Analysis

Stained chromosomes were analyzed after the addition of sodium citrate and sodium sulfite in a dual-beam-flow cytometer as described elsewhere (van den Engh et al. 1985, 1988; Trask et al. 1989). The bivariate flow karyotypes are displayed as contour plots, in which only one contour line at ≈10% of the number of events in the highest peak of the distribution is shown for simplicity. Karyotypes of two individuals were superimposed by rescaling the plots, leaving the positions of chromosome 8 and the origin fixed (van den Engh et al., in press). The positions of the peaks in each karyotype were marked manually using a mouse-driven cursor and a 256 × 256 channel resolution color display on screen to determine the peak coordinates of each chromosome homologue in each individual. Tabulated values were normalized to set the average HO and average CA intensities of all autosomes, except 9–12, at 100. The HO and CA intensities of each homologue were used to derive an estimate of its DNA content. To do this, the relative distance, D_n, between the origin and the projection of the peak for each chromosome on the line running through the origin and chromosome 4 was calculated using equation (1).

$$D_n = HO_n \times \sin \alpha + CA_n \times \cos \alpha , \quad (1)$$

where α is the angle between the x-axis and the projection line ($\tan \alpha = HO_4/CA_4$), and HO_n and CA_n are HO and CA fluorescence intensities of chromosome n. The relative DNA contents were converted to megabasepairs (Mbp) by using an estimate of 3×10^9 bp/haploid genome and the fraction of the genome contained in each chromosome type (Mayall et al. 1984; Trask et al., in press). These values refer to chromosomal DNA contents at G_1.

Results

In figure 1, the bivariate flow karyotypes of two individuals are compared with those of their two daughters. The karyotypes of daughter 1 and her parents are plotted together in figure 2 to give a closer view of chromosomes 9 and smaller. With these flow karyotypes, the parental origin of many chromosome homologues could be determined. The two daughters have each inherited a different set of chromosome heteromorphisms from their parents. *Chromosome 22:* A difference in peak position between chromosome 22 homologues was detected in both parents. The mother's small variant has a lower HO intensity than does the father's small variant. Daughter 1 has inherited the larger variant from both her parents. No peak-position difference between these homologues was detected in her karyotype, as the two homologues together produce a single oval contour line. Daughter 2, in contrast, has inherited her mother's small chromosome 22 and her father's large variant. In her karyotype, the two homologues of this chromosome produce distinctly separated peaks. *Chromosome 21:* Daughter 1 has inherited the smaller of her father's chromosome 21 homologues, and daughter 2 has inherited the larger homologue. Both have inherited the chromosome 21 homologue with higher CA intensity in the mother's karyotype. *Chromosome 19:* The two chromosome 19 homologues carried by the father do not differ significantly in peak position, nor do the mother's chromosome 19 homologues differ in peak position. The karyotypes of both daughters,

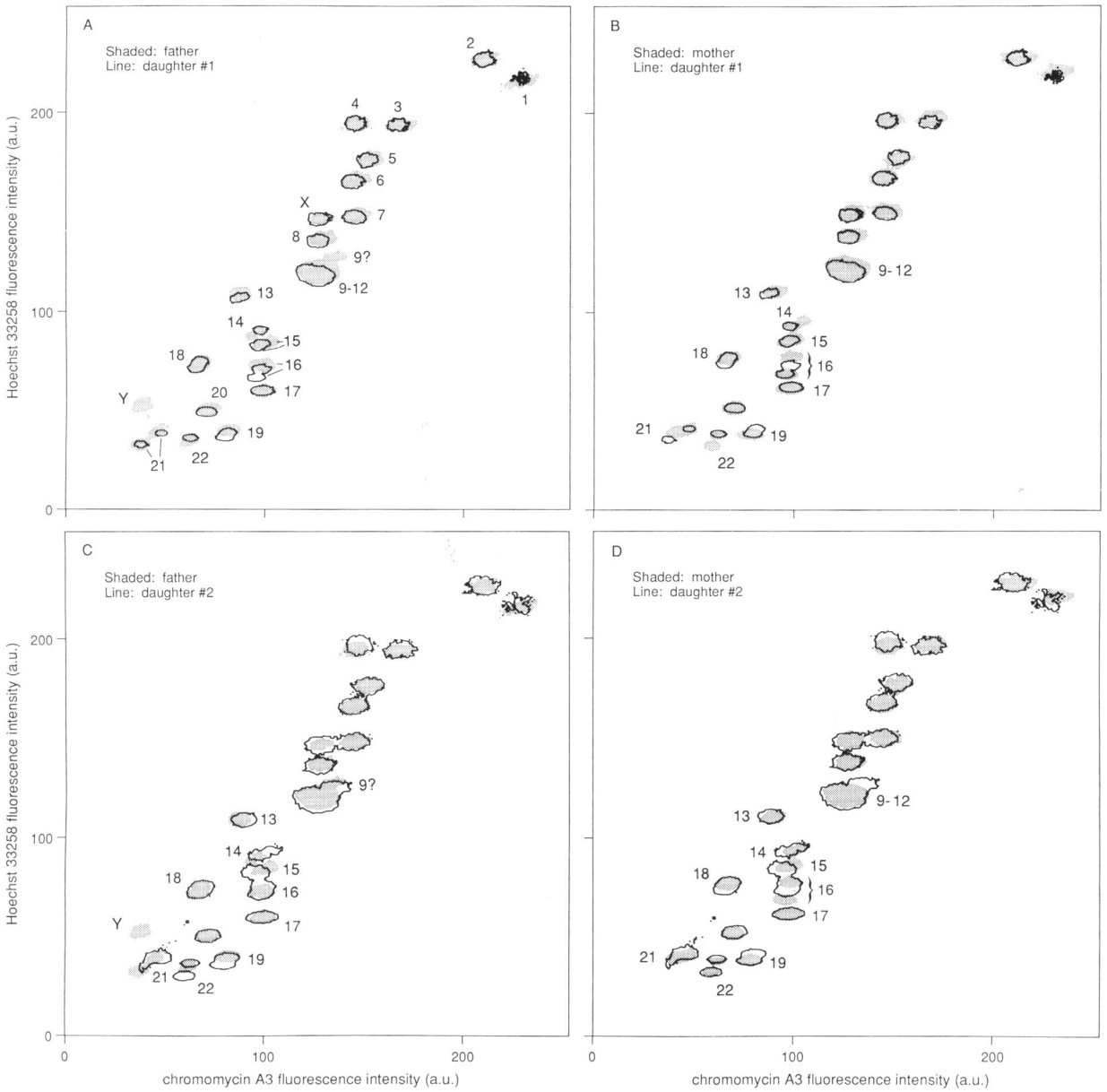

Figure 1 Bivariate flow karyotypes of members of the same family: two daughters and their parents. Chromosomes of all individuals have been stained with HO and CA. The peaks in the flow karyotypes of the father and mother are denoted by the grey shaded areas. The peaks in the karyotypes of the daughters are denoted by the contour lines. *A*, Father (individual 2-1) compared with daughter 1 (2-3). *B*, Mother (2-2) compared with daughter 1. *C*, Father compared with daughter 2 (2-4). *D*, Mother compared with daughter 2. The chromosome type responsible for each peak is identified in panel *A*. The flow karyotype of daughter 1 is shown with more contour levels in fig. 1 of the accompanying paper (Trask et al. 1989).

however, illustrate that the DNA content of the father's chromosome 19 heteromorphism is significantly greater than that of the mother's. The peak representing chromosome 19 is bimodal in both daughters. *Chromosome 18:* A slight peak-position difference between chromosome 18 homologues is visible in the karyotypes of the father and both daughters. Both daughters appear to have inherited the smaller chromosome 18 homologue from their father. *Chromosome 16:* The two homologues of chromosome 16 carried by the mother differ

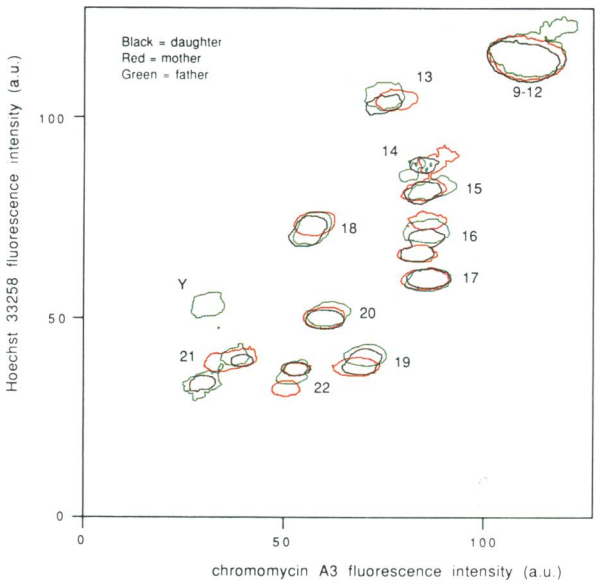

Figure 2 Bivariate flow karyotypes of mother (red lines), father (green lines), and daughter 1 (black lines) of the family also plotted in fig. 1. Only chromosomes 9–22 and Y are displayed.

significantly in HO intensity. The homologues of chromosome 16 are not resolved in the father. Daughter 1 has inherited the small variant of this chromosome from her mother, while her sister has inherited her mother's larger variant. *Chromosome 15:* Neither daughter has inherited the chromosome 15 homologue with higher CA intensity carried by the father. Peak-position differences between chromosome 15 homologues were not detected in either daughter or in the mother. The left side of the chromosome 15 peak does not coincide exactly with the shaded area representing this peak in either parent, suggesting that one of the daughter's homologues may be slightly lower in CA intensity than are those in either parent. *Chromosome 14:* Three different heteromorphisms of this chromosome were observed in this family. The mother carries a chromosome 14 variant with high CA intensity, which has been inherited by daughter 2. The father carries a variant with low CA intensity that has not been inherited by either daughter. An intermediate-sized heteromorphism carried by both parents has been passed from the father to daughter 2 and from both parents to daughter 1. No peak-position difference between the maternal and paternal versions of this heteromorphism is detectable in the latter's flow karyotype. *Chromosomes 9–12:* The father carries a variant of one of the chromosomes (probably chromosome 9) in the chromosomes 9–12 group. Daughter 2 has inherited this variant. Daughter 1 has not. *Other chromosomes:* No significant peak-position differences were observed between homologues of chromosomes 1–8, X, 13, 17, or 20 in members of this family. Thus, the parental origin of a given homologue of these chromosome types cannot be traced with these measurements.

Flow karyotypes of 10 children were compared with the flow karyotypes of their parents to determine the fidelity with which chromosome heteromorphisms are inherited. The results of this analysis are displayed visually in figure 3. Karyotypes of both mother and father were available for nine of the children and of the mother only for one child. The individuals were members of four different families. The peak-position coordinates were determined for each homologue in each karyotype and were normalized as described in Material and Methods. Chromosomes 9–12 were not included in the analysis, because their positions within their combined peak cannot be determined with accuracy. The parental origin for each homologue in each child was determined. One homologue in the child was matched to one homologue in one parent, and the other

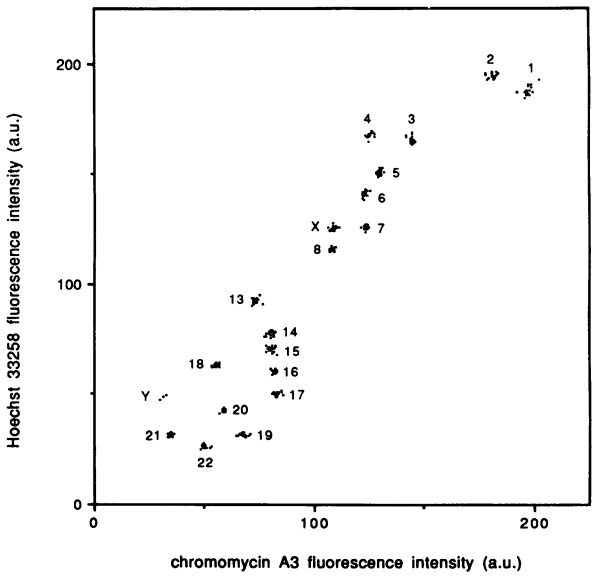

Figure 3 Relative difference in flow karyotype peak position between homologues in children and their parents. Each dot represents the comparison of one homologue in each child to its corresponding homologue in one of its two parents, as described in the text. To produce the figure, karyotypes of 10 children in four families were compared with those of their parents. For each homologue in each child, a child:parent intensity ratio was determined for both HO and CA. These ratios were multiplied by the average HO intensity and CA intensity, respectively, of each chromosome type and were plotted.

homologue in the child was matched to one homologue in the other parents, as described above for the family shown in figures 1 and 2. Of the four possible combinations for each chromosome type, the combination that minimized the sum of peak-position differences between homologues in parent and child was chosen. The HO- and CA-intensity coordinates of each homologue in the child's karyotype were divided by the HO- and CA-intensity coordinates, respectively, of the matching homologue in the karyotype of the parent. Thus, 19 HO and 19 CA child:parent ratios were tabulated for each chromosome type. For each chromosome type, the fractions were multiplied by the average HO or CA intensity measured for 33 unrelated individuals (Trask et al. 1989, table 2) and then plotted in figure 3. The size of the clusters (or the distance of dots to the average peak position) thus reflects in part the fidelity with which homologue peak position is passed from parent to child (see Discussion). The size of the clusters of all chromosome types, including those known to be highly heteromorphic among individuals, is small compared with the size of clusters in unrelated individuals.

The difference in DNA content observed between homologues in parent and child is summarized in table 1. The G_1 DNA content of each homologue type was estimated from its HO and CA intensities according to the protocol described in Material and Methods. The average absolute difference in measured DNA content between homologues in parent and child ranged from 0.4% (chromosome 7) to 2.4% (chromosome Y) and averaged 0.8% over all chromosome types. The basepair equivalent of the average DNA content difference observed between parent and child ranged from 0.5 Mbp (chromosome 16) to 2.0 Mbp (chromosome 1) and averaged 0.95 Mbp. For most of the larger chromosomes, the measured DNA content of a given homologue in a child was within 2% of its DNA content measured in the child's parent. For most of the smaller chromosomes it was within 3%. The largest difference in DNA content observed between a homologue in child and

Table 1

Inheritance of Peak-Position Chromosome Heteromorphisms (10 children, four families)

Chromosome	Average Absolute Difference in DNA[a] Measurement Between Homologue in Child and Parent		Range in Observed DNA[a] Ratios of Child's Homologue Parent's Homologue		Mbp Equivalent[a] of Maximum Observed Difference
	%	Mbp	Minimum	Maximum	
1	.8	2.0	.984	1.022	5.6
2	.5	1.1	.990	1.010	2.5
3	.4	.9	.991	1.012	4.8
4	.6	1.2	.989	1.009	2.3
5	.5	.9	.991	1.013	2.4
6	.6	1.1	.984	1.012	2.9
7	.4	.7	.986	1.004	2.4
8	.5	.7	.991	1.010	1.6
13	.8	.9	.978	1.028	3.1
14	1.1	1.2	.971	1.013	3.1
15	1.0	1.0	.973	1.017	2.8
16	.5	.5	.982	1.013	1.7
17	1.0	.9	.984	1.027	2.3
18	1.1	.9	.974	1.017	2.2
19	1.1	.7	.971	1.032	2.1
20	.9	.6	.972	1.020	1.9
21	1.5	.7	.961	1.024	1.9
22	1.3	.7	.949	1.021	2.7
X	.6	.9	.986	1.013	2.2
Y	2.4	1.4	.976	1.041	2.4
Average	.81	.95			2.6

[a] DNA contents refer to chromosomes in G_1.

parent was 5%. The maximum difference in DNA content observed between homologues in parents and children, averaged over all chromosome types, was equivalent to 2.6 Mbp.

Additional chromosome heteromorphisms can be identified in many individuals if, in place of HO, DAPI is used as the UV-excitable, A-T-binding fluorochrome (Trask et al. 1989). DAPI stains some regions of heterochromatin more intensely relative to other regions than does HO. This has the effect of shifting some chromosomes–particularly chromosomes 1, 9, 16, and Y—to higher positions along the vertical axis in a DAPI karyotype relative to their positions in an HO karyotype. The inheritance of chromosome heteromorphisms identified by their peak position in DAPI versus CA flow karyotypes can also be traced in families, as shown in figure 4. Chromosomes of daughter 1 and her parents were stained with DAPI and CA to produce the bivariate flow karyotypes shown. These are the same individuals whose HO versus CA flow karyotypes are shown in figures 1 and 2. Both chromosome 1 homologues carried by the mother are shifted upward relative to their position in her HO versus CA karyotype (fig. 1*B*), to a position to the right of chromosome 2. In contrast, neither of the father's chromosome 1 homologues shifts significantly on DAPI staining. The karyotype of the daughter shows that she has inherited one variant from her mother that is relatively DAPI bright and one variant from her father that is DAPI dim. These two variants produce separate peaks in her karyotype. Both chromosome 16 homologues in the father are shifted to a slightly higher position along the vertical axis in his DAPI karyotype, relative to their position in his HO karyotype (fig. 1*A*). Only one of the mother's chromosome 16 homologues has shifted relative to its position in her HO karyotype. In the DAPI karyotype, it is at the same position as the peak for chromosome 15. The daughter has inherited the chromosome 16 homologue from her mother that does not shift on DAPI staining. This is the smaller of the daughter's chromosome 16 homologues, in agreement with the results of the HO-karyotype analysis described above. The larger chromosome 16 came from her father, and it also shows a slight shift to a higher position along the vertical axis in her DAPI karyotype relative to her HO karyotype. A result of this is that the peaks for the two chromosome 16 homologues carried by the daughter are more widely separated in her DAPI karyotype than in her HO karyotype. Chromosome(s) in the 9–12 peak (presumably the 9) show a shift to higher UV-excitable

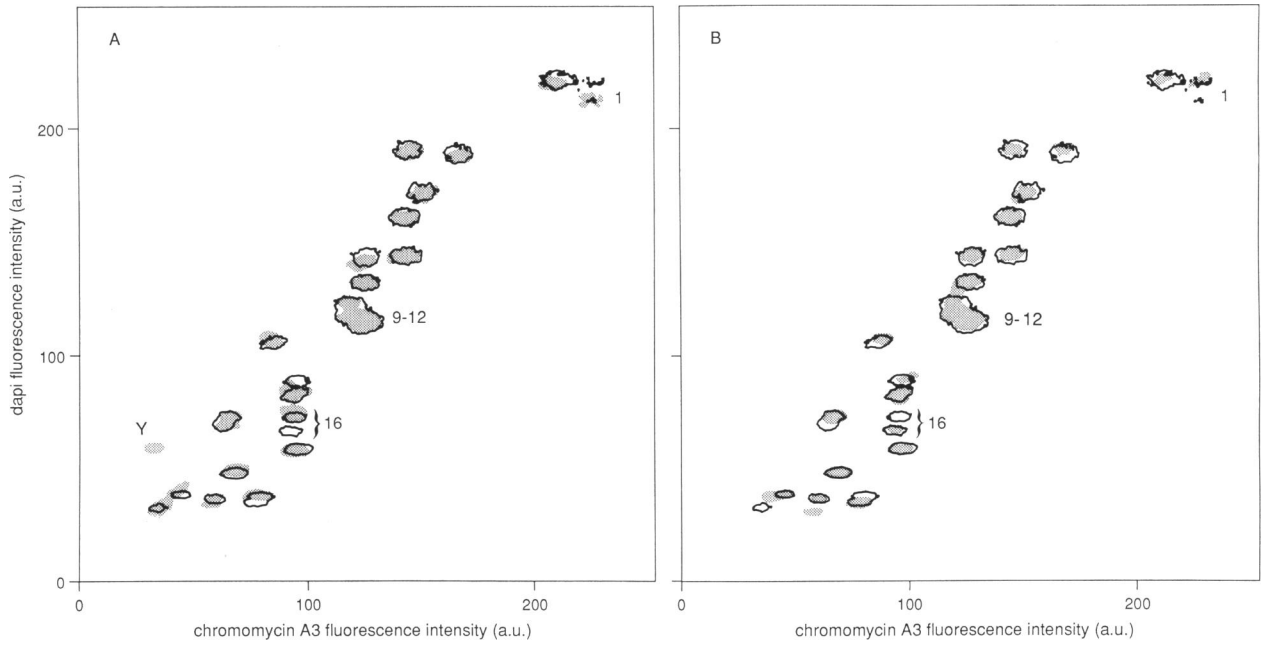

Figure 4 Bivariate flow karyotypes of daughter 1 (individual 2–3) and her father (2–1) and mother (2–2), produced after their chromosomes were stained with DAPI and CA. HO vs. CA flow karyotypes of these individuals are shown in figs. 1 and 2. The peaks in the flow karyotypes of the father and mother are denoted by the grey shaded areas. The peaks in the karyotypes of the daughter are denoted by the contour lines.

fluorescence and to lower CA fluorescence on DAPI staining in all three individuals in this family. The inheritance of heteromorphisms for the remaining chromosomes, which show no significant shifts in peak position on DAPI staining, is in agreement with that described in detail above from the HO versus CA karyotypes in this family.

Discussion

The present study demonstrates that the parental origin of homologues of many chromosome types can be determined by comparing their positions in bivariate flow karyotypes of parents and child. The average difference in G_1 DNA content of a given homologue in parent and child was 0.8%, or 0.95 Mbp, over all chromosome types. The maximum difference in peak position observed was equivalent to a DNA content difference of 2.7 Mbp in chromosome 22. These parent:child differences, or the cluster size in figure 3, reflect the fidelity with which chromosome heteromorphisms are inherited. They are also affected by the accuracy with which peak positions can be determined and with which karyotypes of different individuals can be normalized to each other. Improvements in the latter may allow DNA contents of chromosomes in family members to be compared more accurately than is now possible.

An average of two or three recombination events are estimated to occur per chromosome per meiosis in humans (Alberts et al. 1983). However, chiasma are rarely observed in heterochromatin (Kurnit 1979; Stack 1984). Variants in children have been matched to variants recognized in their parents in most, but not all, studies using conventional banding techniques (McKenzie et al. 1972; Craig-Holmes et al. 1975; McKenzie and Lubs 1975; Robinson et al. 1976; Verma and Lubs 1976; Jacobs 1977; Magenis et al. 1977; Kurnit 1979; Olsen et al. 1986). These observations argue that meiotic recombination within large tracts of repetitive sequences in heterochromatin or in acrocentric satellites may be suppressed relative to meiotic recombination in the rest of the genome. Our data do not contradict these observations. In the small number of families studied (19 comparisons in four families), we have found no evidence of recombination within these regions that has resulted in large differences in the chromosomal DNA content between parent and child. Obviously, detection of recombination within these tracts will require a large number of parent-and-child flow karyotype comparisons. Heterochromatin comprises only 12%–20% of the total DNA content of chromosome 9, 16, or 1, chromosomes with large heterochromatic regions (Podugolnikova et al. 1979), and only some recombinations would be expected to result in a significant DNA content difference between parent and child. Studies by Fitzgerald (1973) and Robinson et al. (1976) suggested that large variants of chromosome 9 may be preferentially inherited. The simplicity and rapidity of flow karyotyping may allow this observation to be corroborated with a larger set of individuals.

Flow karyotyping has proved particularly useful in the detection of submicroscopic deletions in chromosomes of individuals with genetic disorders (Wilcox et al. 1986; Patterson et al. 1987; Cooke et al. 1988; Merry et al. 1989; Schnur et al. 1989; Carter et al., in press; Trask et al. in press) and in human chromosomes in rodent × human somatic cell hybrids (Van Dilla et al. 1986; B. J. Trask, unpublished observations). The sensitivity of deletion detection is limited by the normal variability of some chromosome types, identified in the accompanying paper (Trask et al. 1989). The family studies presented here suggest that flow karyotype information of parents can be informative in the detection of deletions occurring de novo in their child. The abnormal chromosome in the proband is compared not with the distribution of heteromorphisms occurring in the entire population but with any of three parental homologues (the fourth being eliminated by its match to the normal homologue in the proband). A comparison of the cluster sizes in figure 3 in the present paper with those in figure 2*A* in the accompanying paper (Trask et al. 1989) indicates the chromosome types for which parental information is imperative in the detection of small structural abnormalities. For example, while the differences in chromosome 21 DNA content among 33 unrelated individuals can be as great as 19 Mbp, the average and maximum observed absolute differences in chromosome 21 DNA content between parent and child were 0.7 Mbp and 1.9 Mbp, respectively.

Acknowledgments

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